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Research Article

Assessment of Spoilage Microbiota of Rainbow Trout (*Oncorhynchus mykiss*) during Storage by 16S rDNA Sequencing

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Due to the high contents of protein and fat in rainbow trout, it is highly susceptible to spoilage, which limits the storage and transportation processes. Exploring the spoilage microbial community during rainbow trout storage is essential to develop an effective preservation method. Here, the changes in the total bacterial colony and total volatile base nitrogen (TVB-N) during the storage of rainbow trout were investigated. Storage at 0 °C can effectively slow down the spoilage process with bacterial counts and TVB-N contents decreased from 8.7 log CFU/g and 18.7 mg/100 g obtained at 4 °C to 5.6 log CFU/g and 14.5 mg/100 g, respectively. 16S rDNA high-throughput sequencing results showed that the diversity of microbial genera decreased during storage. *Acinetobacter, Pseudomonas,* and *Shewanells* gradually became the dominant spoilage genera with contents of 59.9%, 18.6%, and 1.7%, respectively, in the late stage of storage. The spoilage abilities of bacteria belonging to the *Pseudomonas* and *Shewanells* genera were analyzed. *Shewanella* sp. S5-52 showed the highest level of TVB-N content (100.6 mg/100 g) in sterile fish juice, indicating that it had a strong spoilage ability. This study confirmed the dominant spoilage bacterial genera and evaluated the spoilage abilities of isolated strains during the storage of rainbow trout, which laid the foundation for further investigation of the spoilage mechanism of rainbow trout and other aquatic products.

1. Introduction

Rainbow trout is native to North America and the Pacific Ocean and suitable for growing in low-temperature freshwater, which belongs to the genus Pacific salmon in the family Salmonidae [1]. Rainbow trout can survive under a wide range of temperatures, making it the broadest trout worldwide with high nutritional value and delicious quality [2]. Due to its high protein and fat contents, it is a kind of highly perishable food during storage [3, 4]. Spoilage occurs as a result of a variety of chemical reactions caused by the enzymatic activity of different bacteria, which lead to the quality and economic losses of rainbow trout [5–7].

During the storage of aquatic products, microorganisms play the most critical roles in their spoilage. Detection and identification of the microbial community present in rainbow trout are important to prevent its spoilage and guarantee safety [8]. Using traditional culture-based methods for the identification of spoilage bacteria is time-consuming, and limited information can be obtained [9]. The development of culture-independent 16S rDNA sequencing and classical microbiology methods has contributed to assessing the microbial richness and diversity of various foods and environments [10–12]. The common spoilage bacteria reported include *Pseudomonas*, *Shewanells*, *Psychrobacter*, and *Lactococcus*, which are widely present in various types of marine and freshwater aquatic products [13–15]. *Pseudomonas* spp. are Gram-negative bacteria with a suitable growth temperature of 30 °C and can produce a large amount of protease themselves, so they are often identified as the dominant spoilage bacteria in high-protein foods such as meat and aquatic products [16, 17]. *Shewanells*

is temperature insensitive and can be isolated from stored aquatic products at both room and low temperatures [18]. The spoilage ability of *Shewanells* could be strengthened by sensing exogenous signals through its LuxR receptor, providing theoretical support for the establishment of new technology for aquatic product preservation [19].

Using the 16S rDNA sequencing technology, the main spoilage bacteria have been identified, but the microbial community is highly variable depending on the storage environment and storage [20]. Till now, there is little information about the microbial community of rainbow trout during storage. Therefore, investigating the dynamics and changes of spoilage bacteria associated with rainbow trout during storage is more important to characterize the spoilage process and provide more information for effective preservation methods.

In this study, the effects of different storage temperatures at 0 °C and 4 °C on the quality changes of rainbow trout fillets were investigated by measuring the changes in bacterial colonies and total volatile basic nitrogen (TVB-N) contents. Using the 16S rDNA sequencing technology, we analyzed the changes in the microbial community at 4 °C and determined the dominant spoilage genus during the storage of rainbow trout fillets. Furthermore, a total of 43 spoilage bacteria were isolated, and 6 of them exhibited different spoilage abilities for sterile fish juice.

2. Materials and Methods

2.1. Preparation of Rainbow Trout Samples.
Fresh rainbow trout was purchased from the SM market (Tianjin, China) and transferred to the laboratory within an hour after packaging in a box with ice. The rainbow trout was washed with water and filleted into 60 g of uniform cubical samples. All rainbow trout samples were immediately put into sterilized bags and stored at 0 °C and 4 °C for spoilage monitoring. The microbial and chemical changes of the samples were analyzed on days 0, 3, 6, 9, 12, and 15 of storage.

2.2. Measurement of Colony Number and Total Volatile Basic Nitrogen (TVB-N).
To determine the microbial changes during rainbow trout storage, 10 g of the minced sample was mixed with 90 mL of sterile saline and diluted to prepare a bacterial suspension at the appropriate concentration. The solution was plated on plate count agar and incubated at 37 °C for 48 h. The colony-forming unit (CFU) counts were defined as $\log_{10}$ CFU/g.

The TVB-N contents in rainbow trout fillets were measured according to the previously published method [21]. Briefly, a 20 g of sample was churned and mixed with 100 mL of distilled water, transferred into a bottom flask, and distilled with the addition of 2 g of MgO. A solution containing 3% boric acid (25 mL) and a mixture of 0.1% methyl red and 0.1% methylene blue was used for ammonia titration. The boric acid solution was titrated with sulfuric acid. The reaction was terminated when the color of distillate changed to purple. The quantity of TVB-N was defined as mg/100 g sample and calculated as follows: TVB-N = $(V \times C \times 14 \times 100)$, where V and C refer to the volume and concentration of sulfuric acid, respectively.

2.3. DNA Isolation, Sequencing, and Bioinformatics Analyses.
Samples were collected from rainbow trout fillets, and bacterial DNA was extracted using the cetyltrimethylammonium bromide (CATB) method. The DNA extracts were eluted in 50 μL DNase- and RNase-free water. The concentration and purity were assessed using a NanoDrop spectrophotometer (Implen, CA, USA). For each of the samples, the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGAC-TACHVGGGTWTCTAAT-3′) were used to amplify the V4 region of the 16S rDNA. The PCR conditions were as follows: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 50 °C for 45 s, 72 °C for 60 s, and elongation for 10 min at 72 °C. The PCR products were run on 1% agarose gel electrophoresis and purified using the EZNA Gel Extraction Kit (OMEGA Biotek, USA) according to the manufacturer's instructions. The gel-extracted library was subjected to Qubit quantification and library testing, and sequencing was performed on the Ion S5TMXL platform. Initial filtering and denoising of sequencing results were performed to remove chimeric sequences and obtain the final clean reads. The clean reads were clustered into operational taxonomic units (OTUs) at a threshold of >97% sequence similarity using the UPARSE software [22]. The obtained sequence was submitted to the RDP classifier [23] to obtain the top 10 abundant phyla and genera, respectively. Alpha diversity indicated by the Good's coverage, Shannon, Simpson, Chao1, and Ace indices was evaluated through QIIME.

2.4. Isolation and Identification of Dominant Spoilage Bacteria.
The samples at the end of storage were stirred and diluted with sterile water in a gradient, and 100 μL of the appropriate dilution was incubated on *Pseudomonas* CFC selective agar and triple sugar iron agar at 30 °C for 48 h. Single colonies with different morphologies were selected for purification by scribing, and this procedure was repeated for three times. The purified single colonies were transferred to a 5 mL liquid medium and incubated at 30 °C and 220 rpm for 12 h. Genomic DNA was extracted using a Tguide bacterial genomic DNA kit (OSR-M502, Tiangen Biotech). The bacterial universal primers 27F (5-AGAGTTT-GATCCTGGCTCAG-3) and 1492R (5-GGTTACCTTGT-TACGACTT-3) were used to amplify the genomic DNA. The obtained PCR products with clear bands were sequenced to identify the bacterial species.

2.5. Spoilage Ability Analysis of Isolated Spoilage Bacteria.
Fresh rainbow trout (500 g) was chopped and mixed with 250 mL of distilled water and then autoclaved at 121 °C for 15 min to prepare sterile fish juice. The spoilage bacteria were cultured in LB medium at 30 °C and 220 rpm for 12–18 h to 109 CFU/mL. The bacterial culture was collected by centrifugation at 12 000 ×g for 10 min and resuspended in
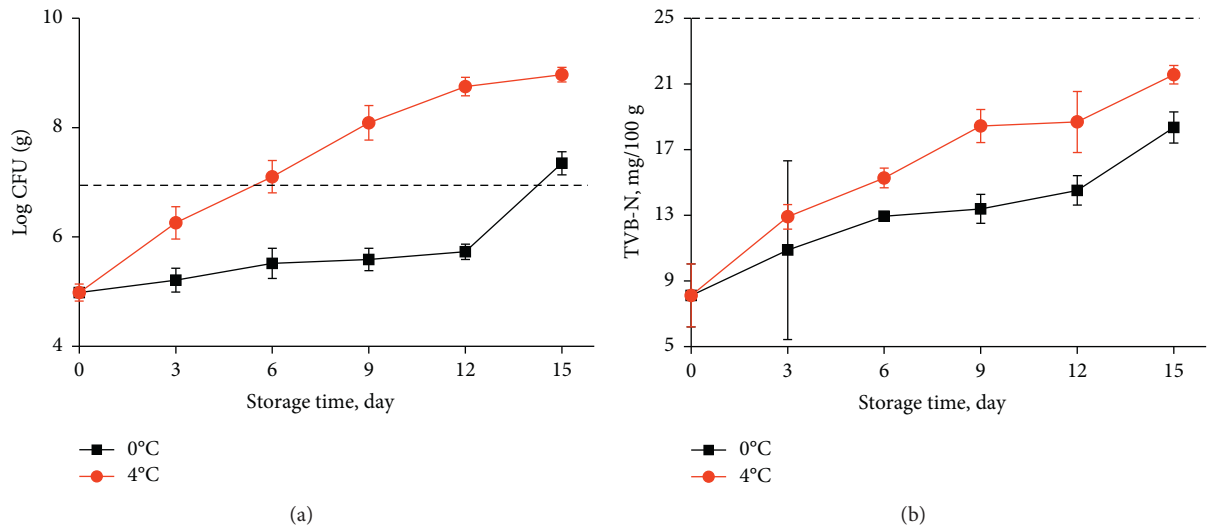
FIGURE 1: Changes in bacterial counts and TVB-N content of rainbow trout fillets during storage at 0 °C and 4 °C. (a) Total bacterial colonies. The dashed line represents the maximum bacterial limit for fish (7.0 log CFU/g) according to the International Commission of Microbiological Standards for Foods (ICMSF). (b) TVB-N content. The dashed line represents the maximum TVB-N limit for fresh fish (25 mg/100 g) according to European Union (EC no. 2074/2005).

stroke-physiological saline solution to obtain the bacterial suspension. For spoilage ability analysis, 20 μL of bacterial suspension was added to 400 mL of sterile fish juice. The total number of colonies and TVB-N content were measured by sampling every 3 days.

2.6. Statistical Analysis. The experimental data were shown as mean ± SD and statistically analyzed using Excel 2010. The one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine significant differences ($p < 0.05$).

3. Results

3.1. Microbial and TVB-N Changes during Rainbow Trout Storage. It is well known that fish spoilage occurs due to bacterial growth during storage, and storage temperature is one of the most predominant factors affecting microbial communities. A temperature range of 0–4°C was commonly used for refrigerator food storage, which could retard the growth of spoilage bacteria [24]. To evaluate the degree of rainbow trout spoilage during refrigerated storage (0 °C and 4 °C), the bacterial numbers were counted at intervals of three days. As shown in Figure 1(a), the initial bacterial counts were 5.0 log CFU/g and gradually increased to 6.3 log CFU/g and 9.0 log CFU/g on days 3 and 15, respectively, at 4 °C. The initial bacterial counts were similar to the results (4.4 log CFU/g) detected by a single nanowire gas sensor in a thermal gradient [25]. On the other hand, the degree of rainbow trout spoilage slowed down at 0 °C during 12 days of storage with bacterial counts of 5.6 log CFU/g, which was far less than that of 8.7 CFU/g obtained at 4°C. However, the bacterial counts rapidly increased to 7.4 log CFU/g in the following 3 days. In comparison, it has been reported that a significant increase in bacterial counts to higher than 10 log

CFU/g was observed within 3 days at room temperature (25 °C) and 6 days at 7 °C [26]. It was indicated that low environmental temperature could effectively inhibit the physiological and biochemical reactions of microorganisms, thus slowing down their growth and spoilage rates. The International Commission of Microbiological Standards for Foods (ICMSF) reported that the maximum bacterial limit for fish was 7.0 log CFU/g. Therefore, the rainbow trout in this study could be stored for 6 days at 4 °C and 12 days at 0 °C. These results suggested that a low temperature of 0 °C enabled prolonged storage time for rainbow trout.

TVB-N produced, when spoilage occurs, is an important parameter to evaluate the degree of food spoilage and widely used as an indicator of the quality of fresh or frozen fish [27]. The TVB-N values of rainbow trout fillets during storage at 0 °C and 4 °C are shown in Figure 1(b). Initially, the TVB-N value was at a relatively low level of 8.1 mg/100 g, which was consistent with the low bacterial counts. During storage at 0 °C and 4 °C, TVB-N contents increased rapidly with increasing storage time. More TVB-N accumulated at 4 °C wit the content 18.7 mg/100 g on day 12, which was comparable with the 18.3 mg/100 g obtained at 0 °C on day 15. The difference indicates that a low temperature can effectively inhibit the production of TVB-N. Fish products were considered unfit for consumption when the TVB-N value reached up to 30.0 mg/100 g [28]. It could be seen that relatively low level of TVB-N was produced during low-temperature storage.

3.2. Alpha Diversity. Due to the demand to maintain the original properties of rainbow trout, 4 °C is a more common preservation temperature [29]. Alpha diversity was used to analyze the relative abundance and diversity of microbial communities within the samples on days 0, 3, 6, and 9 during storage at 4 °C. Rarefaction curves are commonly used to
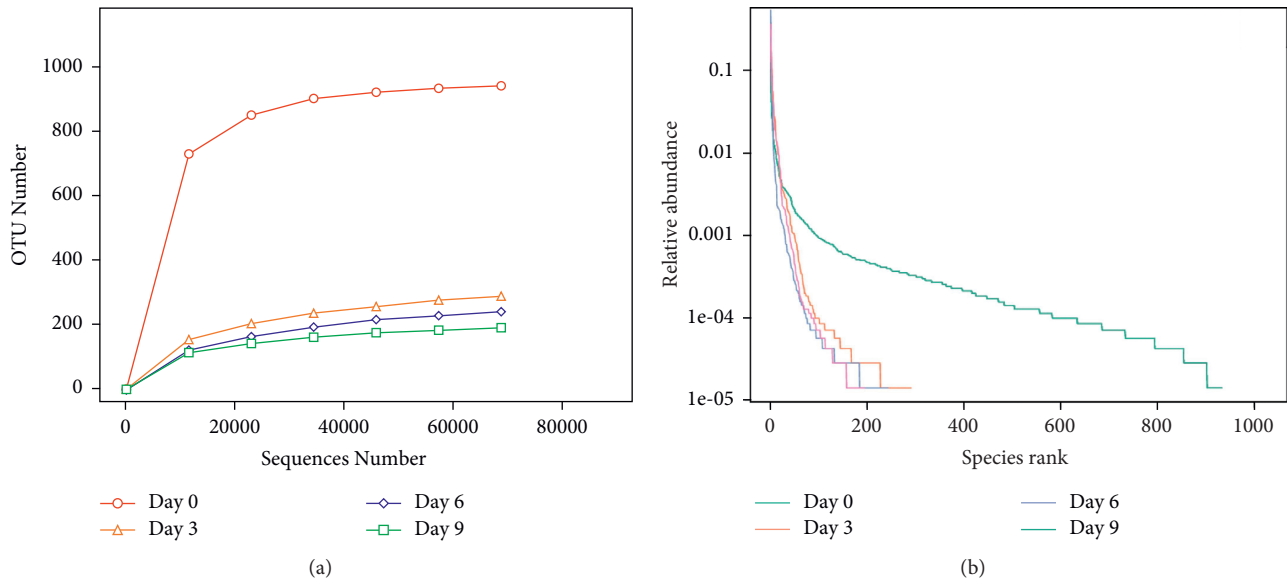
(a)

(b)

FIGURE 2: Rarefaction curves (a) and rank abundance curves (b) of bacterial diversity during rainbow trout fillets storage.

TABLE 1: Diversity index of rainbow trout samples stored at 4 °C for different storage times.

Sample ID	Shannon	Simpson	Chao1	Ace	Goods coverage
Day 0	5.65	0.85	942.49	941.308	1
Day 3	4.227	0.903	324.049	341.392	0.999
Day 6	2.559	0.671	277.396	296.598	0.999
Day 9	3.695	0.832	218.433	224.98	0.999

determine the reasonableness of sequencing data. As shown in Figure 2(a), the rarefaction curves of all samples gradually leveled off with increasing sequence numbers, confirming that the OTUs could represent the overall microbial community. In addition, the rarefaction curves showed that the relative abundance of constituents varied depending on the storage time. The maximum OTU of 933 was observed on day 0 and significantly decreased to 291, 244, and 195 on days 3, 6, and 9, respectively. The rank abundance curves of different storage times also showed that the species composition was richer on day 0 (Figure 2(b)). These results suggested that the OTU number gradually decreased with prolonged storage time.

For alpha diversity analysis, the Shannon, Simpson, Chao1, Ace, and Coverage indices at different storage times are listed in Table 1. The indices of community coverage were all higher than 99.9%, suggesting that sufficient sequencing coverage was achieved. The Chao1 and Ace indices reflected the relative abundance of the community distribution, while the Shannon and Simpson indices represented the diversity of the community distribution. It can be seen that all these indices significantly decreased from day 0 to days 3, 6, and 9 due to the lower richness of bacterial populations during storage. Principal component analysis (PCA) showed that an obvious region discrepancy was observed between the samples stored on different days (Figure S1). Therefore, there were significant differences in bacterial composition among different storage times.

3.3. Spoilage Microbial Community and Predicted Function.
Inhibiting microbial growth and regulating microbial composition could be important ways to prevent quality deterioration. The microbial community during storage of rainbow trout at 4 °C on days 0, 3, 6, and 9 was assessed at the phylum and genus levels.

The bacterial communities of rainbow trout fillets on days 3, 6, and 9 had similar diversity patterns at the phylum level but were different from those of the fresh sample on day 0. As shown in Figure 3(a), a total of 10 dominant bacterial phyla with relative abundance >1% were observed in fresh rainbow trout, including Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes, Tenericutes, Actinobacteria, Cyanobacteria, Verrucomicrobia, unidentified Bacteria, and Acidobacteria. Initially, the dominant phyla were Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes, and Tenericutes, while Fusobacteria and Tenericutes almost disappeared at the end of storage, with relative abundances lower than 0.1%. Proteobacteria developed gradually in the storage process and became the most dominant phylum at the end of storage. Firmicutes was another dominant phylum during storage and was maintained at a relatively high level of 7.6–22.9%. During storage, Proteobacteria and Firmicutes were the most dominant phyla on day 6, with relative abundances of 81.4% and 17.9%, respectively. Since the shelf life of rainbow trout fillets under 4 °C storage was approximately 5 days, it can be tentatively determined that *Pseudomonas* and Firmicutes have a greater influence on rainbow trout spoilage.

Further analysis of the microbial community abundance at the genus level was performed, and the top 10 abundant genera were selected and shown in Figure 3(b). *Pseudomonas*, *Acinetobacter*, *Cetobacterium*, *Brochothrix,* and
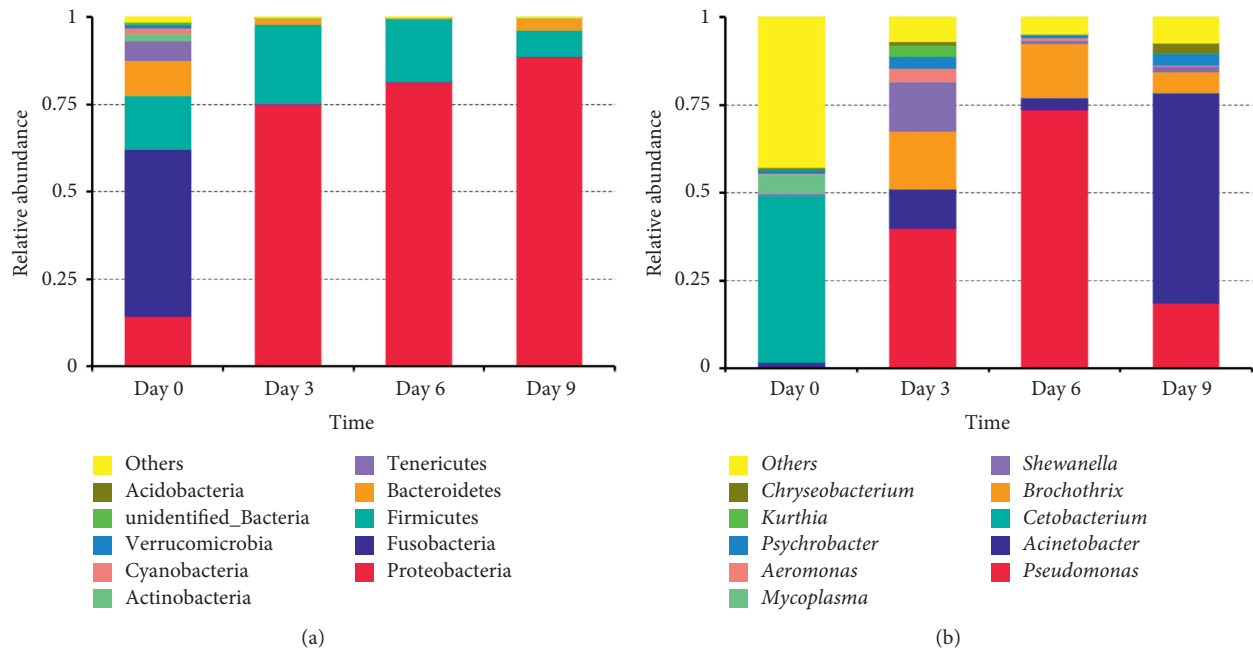
Figure 3: Relative abundance of spoilage microbiota during rainbow trout storage at 4 °C. (a) The phylum level; (b) the genus level.

Shewanella were detected as the most abundant genera in rainbow trout fillet samples. Initially, *Cetobacterium* was the vast majority genus with a relative content of 47.4%; however, it was reduced rapidly to 0.3% on day 6 of storage, indicating that the environment of rainbow trout is not suitable for the growth of this genus. With the extension of storage time, *Pseudomonas*, *Acinetobacter*, *Brochothrix*, and *Shewanella* became the predominant genera with relative abundances >1%. The contents of *Pseudomonas* were 0.5%, 40.0%, 73.8%, and 18.6% on days 0, 3, 6, and 9, respectively, which represented one of the dominant genera during 4 °C storage. An antagonistic interrelationship was observed between *Pseudomonas* and *Acinetobacter*, and the latter was the most abundant genus, with a relative content of 59.9% at the end of storage. The proportion of *Shewanella* was 0.5% at day 0 of storage, first increased to 14.3% at day 3, and then decreased to 1.1% and 1.7% on days 6 and 9, respectively. The results in this study showed that the overall bacterial community was significantly changed during rainbow trout storage. At the beginning of storage, *Cetobacterium* was the most abundant genus, and *Pseudomonas*, *Acinetobacter*, and *Shewanella* constantly showed high abundance during the storage of rainbow trout.

A phylogenic figure of the top 100 spoilage bacterial genera was used to illustrate the system evolution relationship as shown in Figure 4. Except for *Cetobacterium*, the genera with relative higher abundance on day 0 were *Akkermansia*, *Mycoplasma*, and unidentified *Cyanobacteria*, and they had close phylogenic locations. Coincidentally, their abundances were all sharply decreased during storage. Instead, *Pseudomonas* and *Acinetobacter* exhibited close kinship with higher abundance on days 3, 6, and 9. This phenomenon indicated spoilage bacteria with close kinship usually colonized or died out together.

The presumptive functions of the spoilage bacteria are illustrated using PICRUSt and classified by aligning them to the KEGG databases (Figure 5). Three main categories, metabolism, genetic information processing, and environmental information processing, showed relative abundances above 82.0% at different storage times. Among them, the metabolism category was the most enriched function, with a relative abundance above 45.0%. In this category, amino acid metabolism and carbohydrate metabolism showed higher and stable relative abundance in the ranges of 9.5–10.8% and 8.4–11.1%, respectively, during different storage times. It was suggested that metabolites were continuously produced by spoilage bacteria and played a central role in the spoilage process. Nuclear magnetic resonance (NMR) was used for the quantification of metabolites during fish spoilage [30]. Amino acids were identified as the major components in the fish fillets and showed increased trends during storage, which was consistent with the result of our study. Conversely, α-glucose and β-glucose assigned to carbohydrates were gradually decreased with different changing rates. These carbohydrates might be used for the formation of organic acids like acetic acid and lactic acid, which created an unfavourable environment for food storage and were highly associated with spoilage. A relative low level of energy metabolism was observed, indicating energy consumption or generation processes were stable during storage. The following categories were genetic information processing and environmental information processing, which might relate to environmental sensing and signal transduction. It is well-known that quorum sensing plays a vital role in food spoilage, which is tightly associated with changes in the growth environment and signal transduction. Therefore, the functional prediction
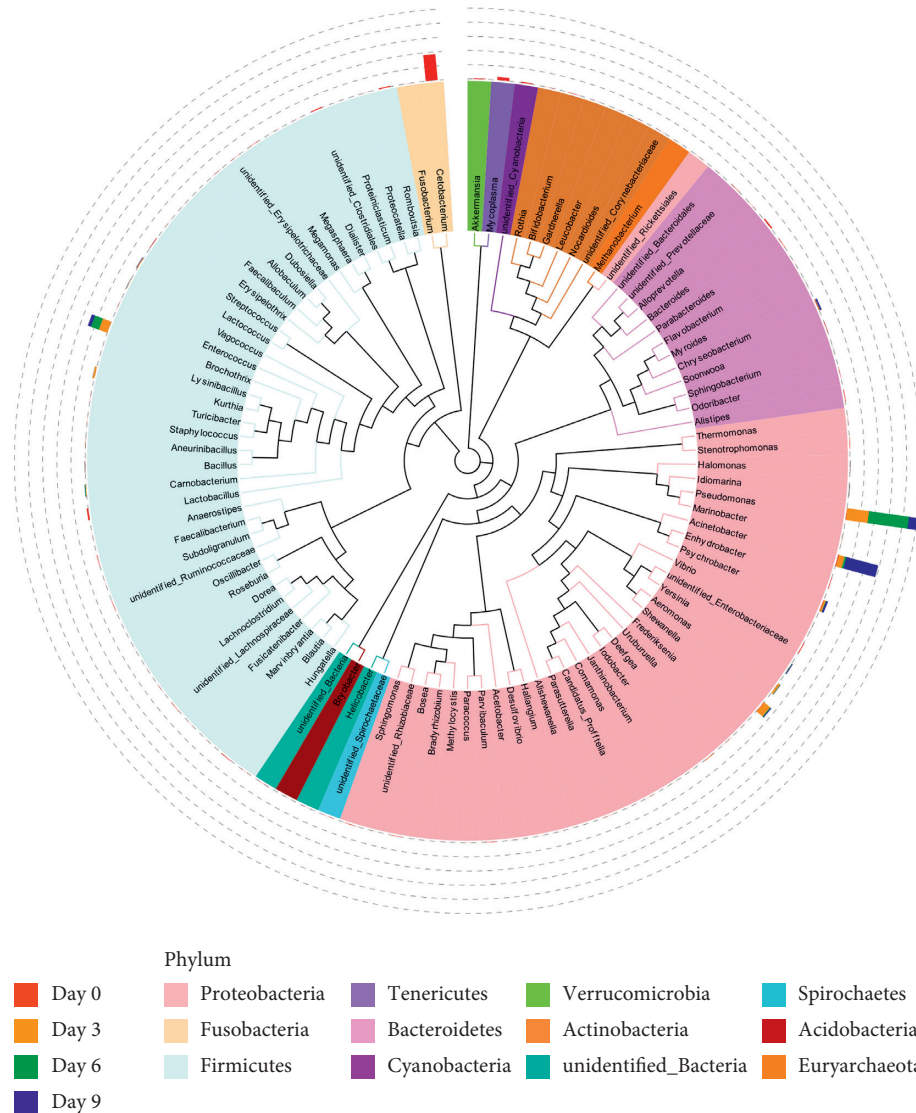
FIGURE 4: Phylogenetic tree based on 16S rDNA sequences on the genus level. Each color of the circle represents a phylum. The branches stand for different genera and the length of the branches indicates the evolutionary distance. The histogram shows the relative reads abundance of different genera.

was beneficial for understanding the influence of bacterial growth and metabolism on rainbow trout spoilage and the spoilage mechanism.

3.4. Identification of Spoilage Bacteria and Evaluation of Spoilage Ability. According to the 16S rDNA sequence, a total of 43 spoilage bacteria were isolated through a culture-dependent approach (Table S1). As *Pseudomonas* and *Shewanella* were the most abundant genera, 4 *Pseudomonas* strains, including *Pseudomonas fluorescens* KBL29, *Pseudomonas putida* DSQ4, *P. fluorescens* CP DA19, and *Pseudomonas fragi* Sneb811, and 2 *Shewanella* strains, including *Shewanella* sp. S05 and *Shewanella* sp. S5-52, were cultured in sterile fish juice to evaluate their spoilage ability by measuring the changes of total colony numbers and TVB-N contents during storage. As shown in Figure 6(a), there were no clear distinguishable patterns among the 6 isolated

strains in colony numbers. Both the 2 *Shewanella* strains showed high bacterial counts in the later stages of storage. Correspondingly, the strain *Shewanella* sp. S5-52 accumulated the highest level of TVB-N, with the content increasing from 14.9 mg/100 g on day 0 to 100.6 mg/100 g on day 12 (Figure 6(b)). *P. putida* DSQ4 also showed a higher TVB-N content of 73.3 mg/100 g. It was tentatively determined that these two strains had a strong spoilage capacity. In contrast, the accumulation of TVB-N on day 12 in *P. fragi* Sneb811 was the same as on day 0 with no more production and accumulation of TVB-N during the storage period, indicating that this strain had no spoilage ability.

4. Discussion

Environmental factors, including storage temperature, pH, oxygen, and environmental bacteria, are important in the storage of rainbow trout over time. Among these factors,
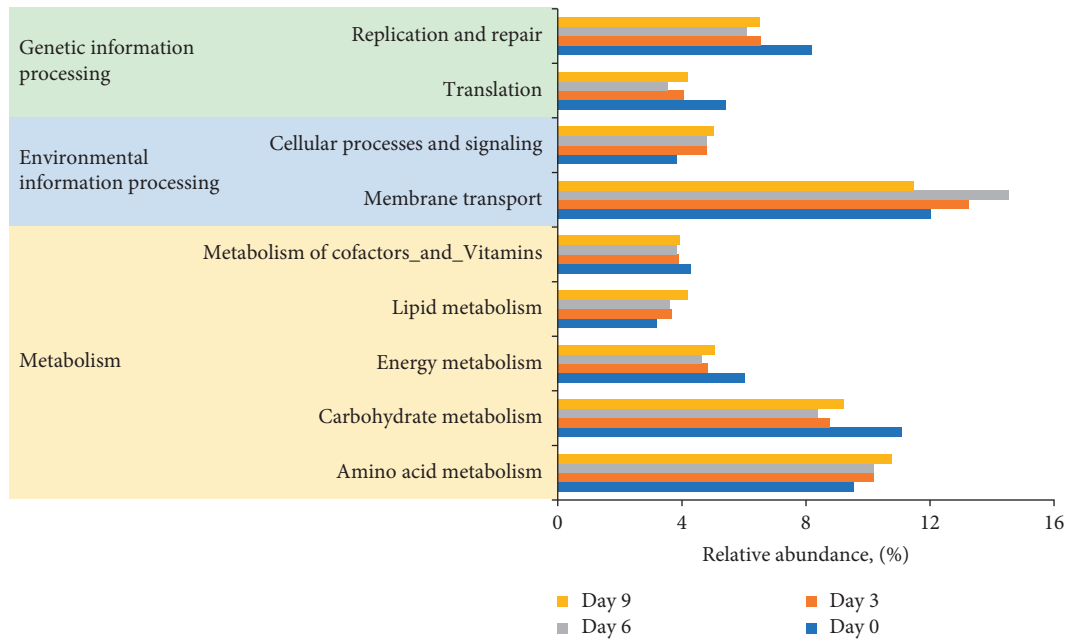
FIGURE 5: Presumptive functions of spoilage bacterial KEGG categories (level 2) during rainbow trout fillet storage.
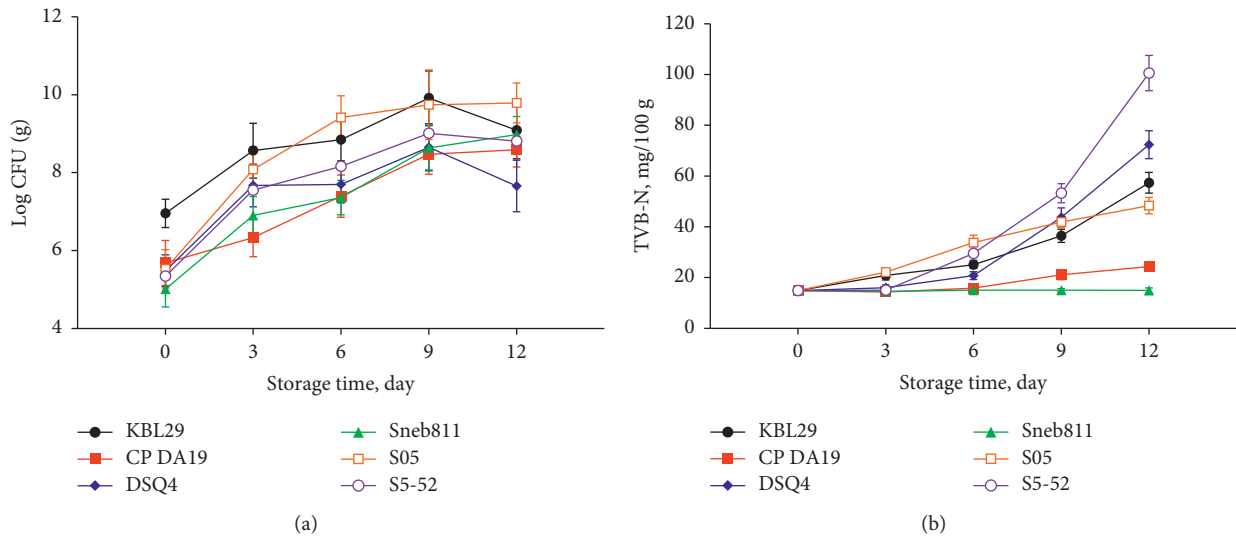

FIGURE 6: Changes of total bacterial colonies and TVB-N during the storage of rainbow trout juice incubated with 6 isolated spoilage strains. (a) Total bacterial colonies; (b) TVB-N.

storage temperature plays a predominant role in fish spoilage by affecting the bacterial cell growth rate, the duration of the lag phase, and the enzymatic activity. Generally, different storage temperatures can result in different effects on the spoilage process of fresh fish [31]. It has been proven that low-temperature storage enables the inhibition of spoilage strain growth and a reduction in TVB-N content, thus extending the shelf life of fish flesh during storage [31, 32]. However, it should be noted that freshness is an essential aspect of aquatic products, and frozen temperatures below 0 °C could be detrimental for maintaining the microstructure and nutrition of food [33, 34]. As the demand

for fresh foods increases, a suitable storage temperature toward extending the shelf life is necessary. We reported the changes in spoilage bacterial numbers and TVB-N content during storage of rainbow trout fillets at refrigerated temperatures (4 °C) and 0 °C. It was suggested that a low storage temperature at 0 °C could slow down the rate of bacterial growth and the generation of TVB-N, thus extending the shelf lives of rainbow trout. This study provided evidence that the spoilage process of rainbow trout fillets is highly temperature dependent.

High-throughput sequencing technology has been widely used for assessing microbial community during food

spoilage. By using this technology, *Pseudomonas*, *Aeromonas*, *Shewanella*, and *Acinetobacter* were commonly found during a variety of aquatic products storage at low temperature [35–37]. Our study showed that the *Pseudomonas* and *Shewanella* were most abundant during rainbow trout storage, which was similar to the microbial community during refrigerated storage of seabass fillets [38]. Compared with other bacteria, *Pseudomonas* usually shows a high spoilage activity, thus the increase of *Pseudomonas* during storage indicates a rapid spoilage process [39]. It has been widely accepted that *Shewanella* is the dominant spoilage genus during fish storage due to its high tolerance to environmental changes [40–42]. *Shewanella* is a genus with a high sulfur production capacity during cell growth [43]. At the same time, the ability to form higher levels of biofilm indicates that it has a strong adhesion capacity and is more likely to adhere to the surface of aquatic products [15, 44]. Therefore, *Shewanella* can gradually become dominant in rainbow trout during storage and plays a significant role in the spoilage process.

High-throughput sequencing can almost completely cover the species in rainbow trout samples, reflecting the microbial composition of each storage period [45, 46]. However, it is not possible to effectively analyze the spoilage ability of specific bacteria. The use of the traditional culture isolation method and subsequent sequencing analysis can provide more accurate information on the spoilage ability of specific strains. The two methods have their own advantages and should be combined to provide a deeper understanding of the spoilage mechanism and process [31]. The strains belonging to the *Pseudomonas* genus showed different contents of TVB-N, indicating their different spoilage abilities. Unexpectedly, despite the absolute predominance of *Pseudomonas* in the microbial community, a strain belonging to *Shewanella* exhibited the highest TVB-N content. The TVB-N is generated by the protein degradation, which is related to the endogenous enzyme activity [47]. The latest study revealed that myofibril degradation was highly associated with the proteolytic effect of endogenous enzymes, which might result in the disassembly of myofibril filament [48]. Furthermore, protein degradation can provide additional carbon sources and energy for spoilage bacteria by compensated glycolysis and amino acid metabolism [49]. As a result, TVB-N content often increases with prolonged storage time. Antimicrobial edible coating of fish gelatin, tea polyphenol, and chitosan has been shown to be effective in preserving protein integrity, thus preventing protein degradation and spoilage during cold storage [50, 51].

5. Conclusions

In conclusion, we found that storage at 0 °C can extend the shelf life of rainbow trout fillets, and a faster spoilage rate is observed at 4 °C. From 16S rDNA analysis, *Pseudomonas*, *Acinetobacter*, and *Shewanella* were the most abundant spoilage genera. Combined with the traditional culture-dependent method, a strain belonging to the *Shewanella* genus was isolated and showed strong spoilage ability to rainbow trout juice.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest. here comes

Authors' Contributions

Guangqing Du and Yuanming Gai contributed equally to this work.

Supplementary Materials

Supplementary data associated with this article can be found in the online version. (*Supplementary Materials*)

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